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5. THE ROLE OF LIPIDS IN THE DEVELOPMENT OF ATHEROSCLEROSIS AND CORONARY HEART DISEASE: GUIDELINES FOR DIAGNOSIS AND TREATMENT

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5.1 General aspects

Cholesterol was recognised as the lipid present in the atheromatous plaques in the 19th century soon after its discovery [1]. The epidemiological association between serum cholesterol or more precisely serum low-density lipoprotein (LDL) and coronary heart disease (CHD) was well established by the 1960s and the confirmation in the 1970s that familial hypercholesterolaemia was a monogenic disorder due to mutations of LDL receptor demonstrated that raised circulating LDL without the need for other CHD risk factors could cause accelerated atherosclerosis [2, 3]. It was also demonstrated that the cholesterol in atheromatous lesions was derived from LDL cholesterol. Also in the 1970s it was recognised that low levels of high-density lipoprotein (HDL) were a potent risk factor for atherosclerosis often more important even than LDL in women and older patients [4]. Raised serum triglyceride levels have only recently become recognised as risk factors for CHD. Earlier controversy about triglycerides and CHD risk may have been the consequence of the greater biological variation in the serum triglyceride concentration compared to HDL cholesterol with which they are relatively strongly correlated, which meant that triglycerides were rejected in multivariate analysis of CHD risk on mathematical grounds.

Despite the knowledge that LDL is involved in atherogenesis, its exact role was until recently poorly understood. Although macrophages were identified as the principal cell type that gave rise to lipid-laden foam cells in fatty streaks and mature atherosclerosis lesions, macrophages in tissue culture displayed little capacity to take up LDL. Indeed, macrophage LDL receptor expression is low compared to other cell types such as fibroblasts. This conundrum was solved when it was found that chemically modified LDL (acetylated LDL) could be rapidly taken up by receptors on macrophages, which were not down-regulated, as increasing amounts of cholesterol entered their cytoplasm so that foam-cell formation occurred. Oxidation of LDL

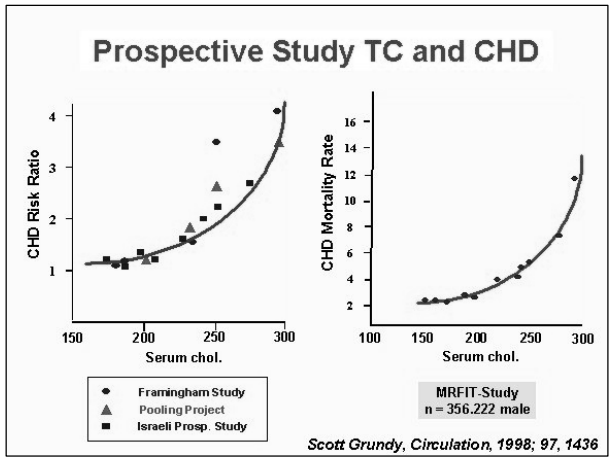

Figure 1. Serum cholesterol and CHD risk

(LDLox) could occur in the biosystem and was found to produce similar rapid uptake of LDL by macrophages through the acetyl-LDL receptors and through other classes of receptors.

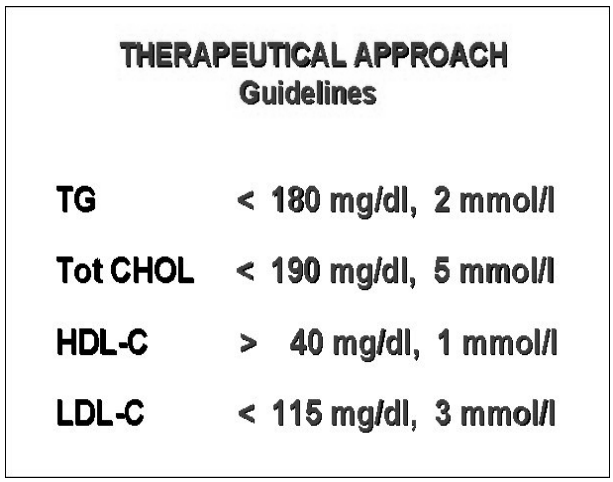

Figure 2. Therapeutical approach guidelines accepted by the European Societies for Cardiology, Hypertension and Diabetes

From_epidemiological and prospective studies a qualitative and a quantitative relationship between CHD risk ratio, CHD mortality rate and serum cholesterol concentration was dedicated (Figure 1). Guidelines, by task forces of NCEP (National Cholesterol Education Program) and of

the EAS (European Atherosclerosis Society), of diagnostic and therapeutical approach were developed and accepted by the European Societies for Cardiology, Hypertension and Diabetes (Figure 2).

Half of all myocardial infarctions occur in persons in whom plasma lipid levels are normal. In an effort to better identify patients at high risk for cardiovascular events, several markers of risk have been proposed for use in screening, including homocysteine and fibrinogen levels, fibrolytic capacity and levels of apolipoprotein A-I, apo B-100 and Lp (a).

5.2 Lipoprotein metabolism

The reference method for separation of blood lipoproteins is still the analytical ultracentrifugal method, where Gofman and Lindgren developed a method based on density (g/ml) (Figure 4).

The electrophoretic technique according to Frederickson creates the phenotype classification of the dyslipoproteinaemia. However, it contains great genotype variation. The actual classification is based on the

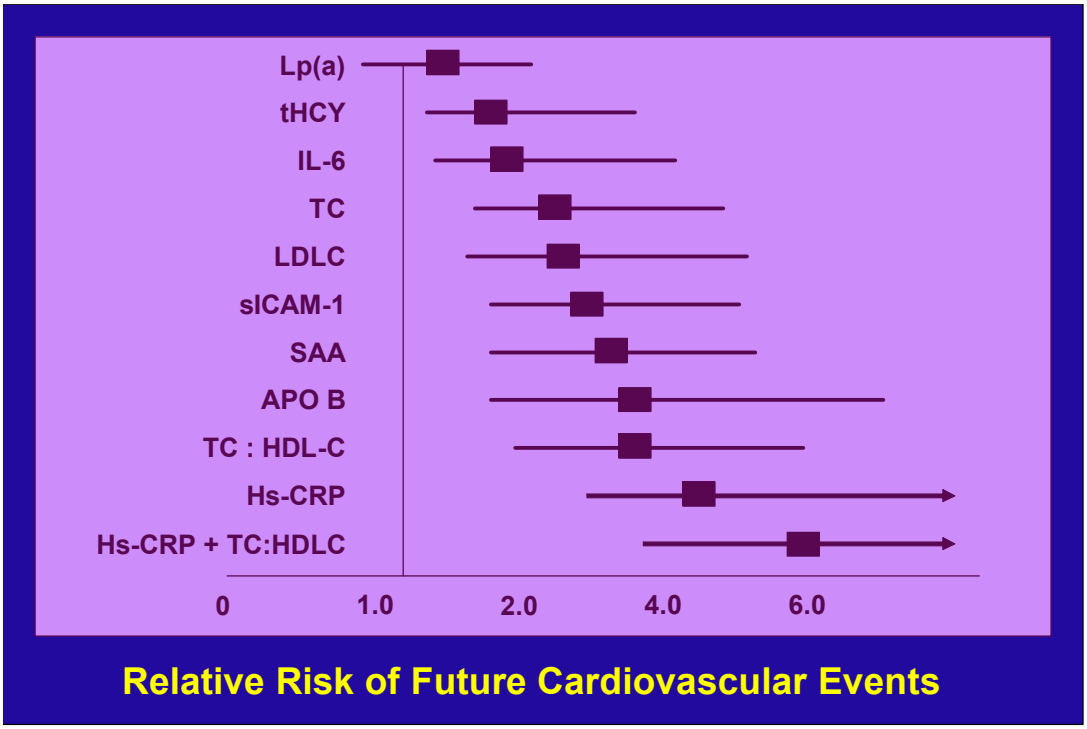

Figure 3. Relative risk of future cardiovascular events

With the recognition that atherosclerosis is an inflammatory process, several plasma markers of inflammation have also been evaluated as potential tools for prediction of the risk of CHD events. Among them are markers of systemic inflammation produced in the liver, including high sensitive C-reactive protein (hs-CRP), serum amyloid A, cytokines such as interleukin 6 and soluble intercellular adhesion molecule type I (I CAM-I).

In Figure 3 the relative risk of future cardiovascular events is described. Hs-CRP combined with the TC/HDL-C ratio is the most pronounced risk factor for CHD [4]

biological values of the patients: hypercholesterolaemia, hypertriglyceridaemia and mixed hyperlipidaemia. The primary hyperlipidaemia based on a genetic deficiency is classified according to biochemical mechanisms.

In Figure 5, we describe the pathway of lipoprotein metabolism. Synthesis of liver lipids and lipoproteins lead to the formation of the lipoprotein cascade and possible to the formation of LDL-oxidised particles which are taken up by SR-A (scavenger receptor) on the macrophages or by SR-B receptors on the liver. There is also a well-known interaction between HDL-Lp and low-density lipoproteins where three protein enzymes play an important role for lipid exchanges: LCAT, lecithin-cholesterol acyltransferase; CETP, cholesterol ester transport protein; and PTP, phospholipid transport protein.

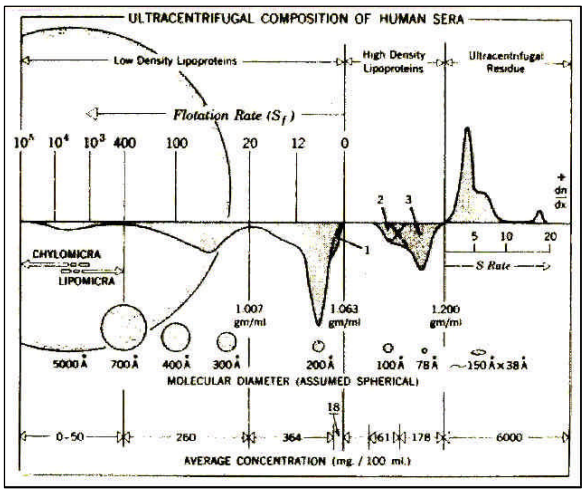

Figure 4. Ultracentrifugal composition of human serum

More attention is paid to the lipoprotein subclasses, which are better known. Chylomicrons (d< 0.94 g/ml) are from exogenous source and are rapidly metabolised through LpL. If dysfunction of the enzyme is present, chylomicron remnants are formed, which are atherogenic. VLDL (very low-density lipoprotein) (0.9> < d> 1.006) secreted by the liver has different pathways regulated by LpL and cell receptors. VLDL-1 has two metabolic ways, the major part is taken up by the cell receptor, the minor part is further metabolised via LpL and apo C-II as cofactor to VLDL-2.

VLDL-2 is further degraded to LDL and taken up by apo B / E or apo B receptors to be catabolized. VLDL remnants due to enzyme dysfunctions are also atherogenic. LDL particles are low-density lipoproteins (1.019< d < 1.060 g/ml) and are composed from three major fractions LDL I, 1.020 < d< 1.035; LDL II, 1.035 < d< 1.045 g/ml and LDL III, 1.045 < d< 1.060 g/ml, and are direct related to the plasma triglyceride levels. The small LDL particles have a very low affinity to the cell receptor, are well oxidised and highly atherogenic.

High-density lipoproteins with density range 1.063 < d< 1.02 are protein rich. Three main subgroups are identified as pre-Beta HDL, HDL2 and HDL3. The interconversion is determined by exchange of lipids and lipolytic mechanisms including the LCAT activity. The size of the particles is related to the TG plasma levels.

The regulation of HDL subfraction distribution is given in Figure 6. Plasma triglyceride-rich (TG-rich) lipoproteins, CETP and HDL influence the HDL2 /HDL3 distribution in a manner similar to that of LDL subfractions. It is postulated that HDL3 can be converted back into HDL2 when phospholipid (PL) and free cholesterol (FC) , from cell membranes or from the surface of triglyceride rich lipoproteins undergoing lipolysis, are integrated into the particle. LCAT action expands the hydrophobic core with CE to generate larger HDL2 particles.

APO A (I) , shed either from shrinking HDL particles or from the surface of chylomicrons, or produced by de novo synthesis, is believed to interact with phospholipid and free cholesterol to form a small, precursor HDL particle which has pre-Beta migration on electrophoresis. LCAT action can convert this particle into mature HDL.

5.3 Clinical significance of lipoprotein subclasses

Although high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels have long served as the primary indicators of risk for coronary heart disease (CHD) , their diagnostic accuracy is limited. In fact, about half of all individuals who develop heart disease have “normal” HDL and LDL cholesterol levels, and many people with “adverse” cholesterol levels do not develop CHD.

The most common and well-characterised lipoprotein metabolic risk is termed the atherogenic lipoprotein phenotype (ALP) . Present in almost 50% of men with heart disease, the ALP is characterised by an over-abundance of particles of the small, dense LDL subclass in the circulation. People with the same LDL-C level can have LDL particles that are predominantly large (LDL-subclass pattern A) or small (LDL-subclass pattern B) , depending on their metabolic circumstances. Those with mainly small LDL particles are likely to have elevated triglyceride levels and low levels of HDL in the larger subclasses, which are additional characteristics in the ALP.

The techniques used most frequently for subclass fractionation include various types of ultracentrifugation, electrophoresis, chemical precipitation and chromatography. They often take several hours to several days to complete and usually achieve only partial resolution of the subclasses. It might be possible simultaneously to quantify a large number of lipoprotein subclasses without employing physical fractionation of the plasma. Proton NMR spectroscopy difference exhibited by lipoprotein particles of different sizes is a new process and a dedicated intermediate-field (360 MHz) NMR analyser is used.

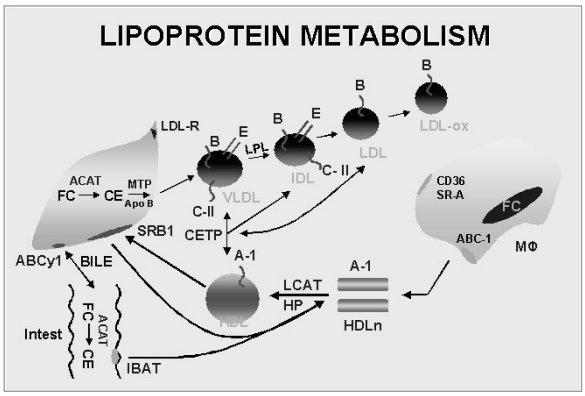

Figure 5. Pathways of lipoprotein metabolism

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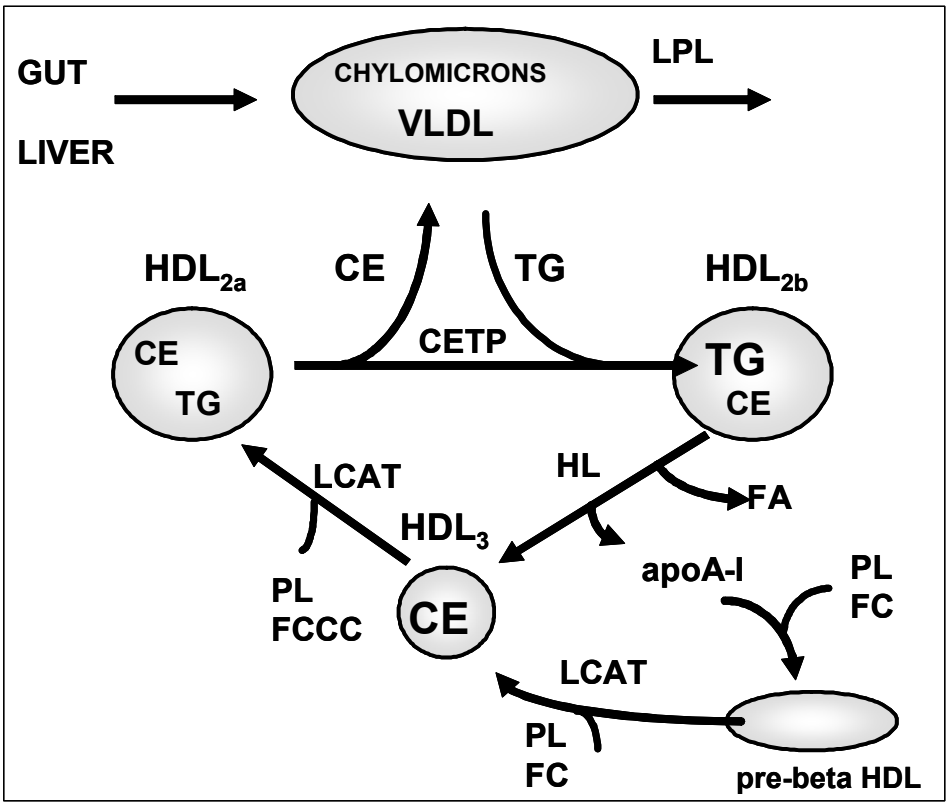

Figure 6. Regulation of HDL subfraction distribution

There is, for example, abundant evidence that LDL particle size is an important determinant of CDH risk (1). Several cross-sectional and prospective studies have shown that individuals with predominantly small, dense LDL particles (subclass pattern B) are at increased risk for CHD even when levels of LDL-C are not elevated. Differing associations of HDL subclasses with CHD have also been noted. Of the five subclasses separable by gradient polyacrylamide gel electrophoresis (PAGE), the three largest (HDL2b, HDL2a and HDL3a) show the expected inverse correlation with disease incidence and severity, whereas the two smallest subclasses (HDL3b and HDL3c) show a positive association.

Thus, for the same reason that TC is often an unreliable indicator of CHD risk, HDL-C levels might not accurately predict the degree of CHD protection.

The NMR lipoprofiles of two middle-aged men (patients A and B) illustrate how different the underlying metabolic status and associated risk of CHD can be for two people who have virtually identical LDL and HDL cholesterol levels (Figure 7). In figure we have an example of the type of useful information provided by the NMR Lipo Profile concerning individual responsiveness to treatment.

A method more applicable for clinical work is gradient gel electrophoresis were the presence of two main patterns for LDL subfractionation is described: pattern A, in which large LDL predominated; and pattern B, where small LDL was the major species. In Figure 8 regulation of LDL subfractionation distribution on PAGE is described.

LDL in most individuals exists as three discrete species. In those with low plasma triglyceride levels (0,5- 1,3 mmol/l), larger species (LDL-I and LDL-II) are most abundant (giving pattern A on gradient electrophoresis), while subjects with high normal triglyceride (> 1,5mmol/l) have a predominance of small, dense LDL-III (pattern B). It is postulated that LDL I/II is converted into LDL-III by cholesteryl-ester transfer protein (CETP-mediated exchange of LDL cholesterylesters (CE) for triglyceride from triglyceride-rich proteins and subsequent action of hepatic lipase, which lipolyses the triglyceride-enriched LDL, leading to the formation of a smaller dense species).

Smaller LDL has a lower affinity for LDL receptors than its larger counterparts. The metabolic scheme also predicts the action of lipid-lowering drugs on the LDL subfractions. Agents which stimulate LDL receptor activity are likely to promote the clearance of LDL-I and LDL-II, as has been observed. Plasma triglyceride-lowering compounds on the other hand, shift the patterns from smaller to larger LDL-species, as has been seen with fibrates.

5.4 The metabolic syndrome

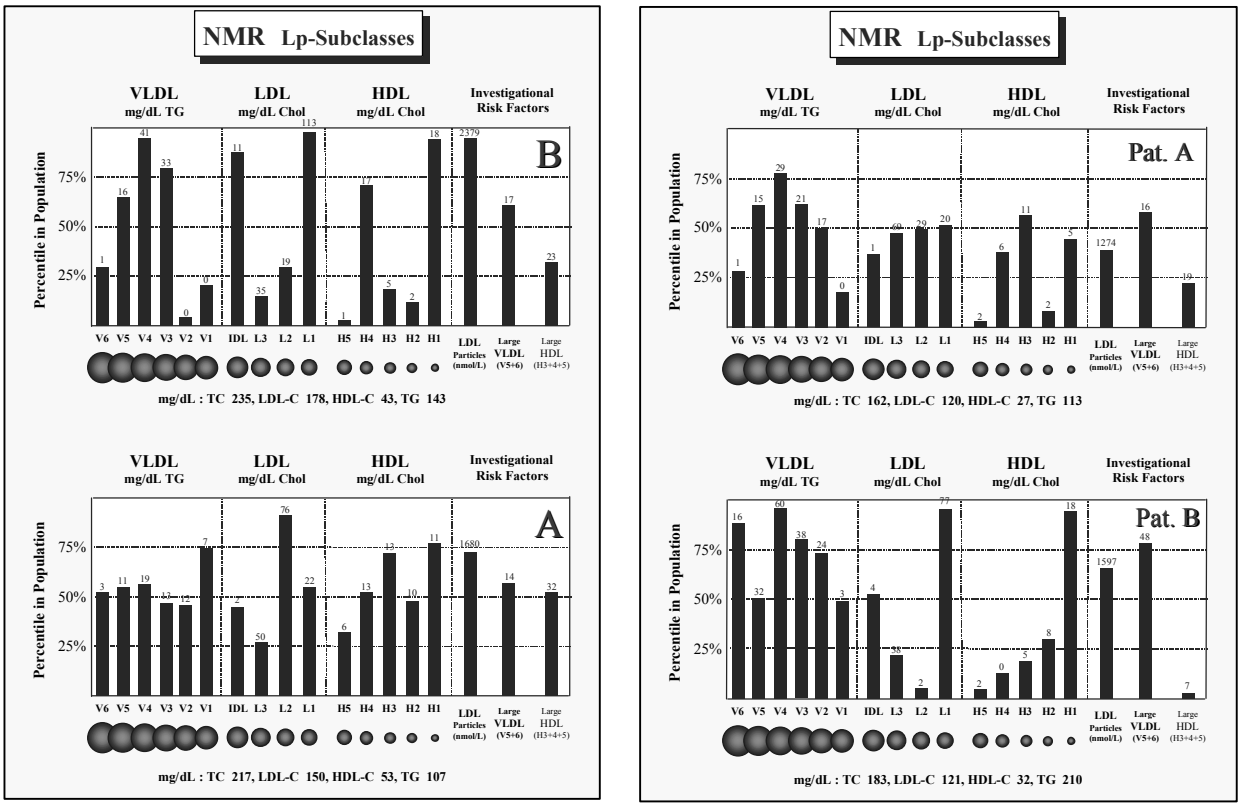

Figure 7. The NMR lipoprofiles of two middle-aged men (patients A and B)

Insulin resistance syndrome is often encountered in a series of clinical situations [5, 6]. In everyday practice its most frequent form is that know as syndrome X or plurimetabolic syndrome. It consists of several metabolic abnormalities. All of them are recognised as independent cardiovascular risk factors, especially for coronary artery disease and stroke. The mechanism relating insulin resistance and dyslipidaemia are given in Figure 9. Over production of glucose and triglycerides lead to formation of small LDL and HDL particles. We consider here the influence of low HDL levels and small HDL particles on coronary heart disease.

There is no doubt that low levels of HDL-C are associated with an increased incidence of cardiovascular events. Multiple mechanisms may explain how HDL slows progression of atherosclerosis and retards the development of CHD.

HDL encompasses heterogeneous classes of lipoproteins which have in common a high density (> 1.063g/ml) and a small size (Stoke's diameter 2 to 17mm). The majority of the HDL particles contain apo A-I. Differences in the quantitative and qualitative content of lipids, apolipoproteins, enzymes and lipid transfer proteins result in the presence of various HDL subclasses characterised by differences in shape, density, size, charge and antigenicity.

Pathways involved in the generation and conversion of HDL are discussed. Among several causes explaining insulin resistance, it has been speculated that it may be mediated in part by an increase in free fatty acids (FFA), which inhibits postinsulin receptor signalling and contributes to insulin resistance. As resistance to insulin action or insulin deprivation is associated with increased lipolysis, intra-abdominal fat, which is metabolically very active, releases FFA into the portal circulation.

The liver converts FFA into triglycerides and may explain the hypertriglyceridaemia associated with the plurimetabolic syndrome. The rise in concentration of TG-enriched particles leads to a reciprocal exchange of FA: CE to VLDL and chylomicron remnants, while TG are transferred to LDL and HDL particles to form small dense LDL and HDL, which are well known for their atherogenic potential.

The low HDL-C syndrome, one factor of the metabolic syndrome, often occurs with other risk factors. Most patients with low levels of HDL also have high triglycerides, a high proportion of small dense LDL-C particles and elevated levels of highly atherogenic chylomicron remnants. These patients are often obese and frequently have a highly degree of insulin resistance, a hyperinsulinaemia, increased concentrations of plasmogen activator inhibitor (PAI) and abnormal postprandial lipaemia.

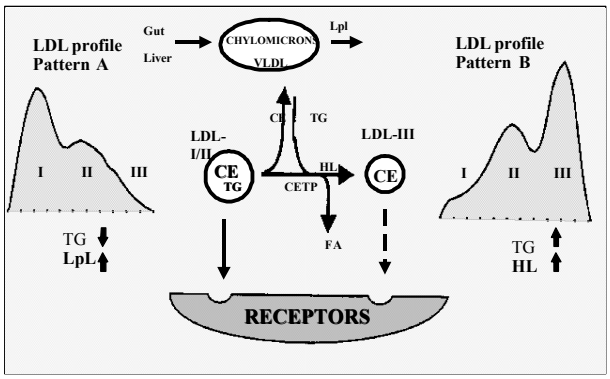

Figure 8. Gradient gel electrophoresis

As a consequence therapeutic modifications of HDL-C levels have attracted considerable interest and drugs increasing HDL are sought for antiatherogenic therapies. A meta-analysis of four large prospective studies has defined the relationship between changes in HDL and shifts in CV risk. An increase of 0.26mmol/l reduced the incidence of events by 2% in men and 3% in women. The protective functions of HDL may explain how the molecule limit inflammation and mitigate atherogenesis.

It has to be remembered that insulin resistance syndrome and type 2 diabetes are two pathological conditions closely linked to the "Western Way of Life". It would be worth trying to first modify lifestyle by changing dietary physical and rhythm-of-work habits.

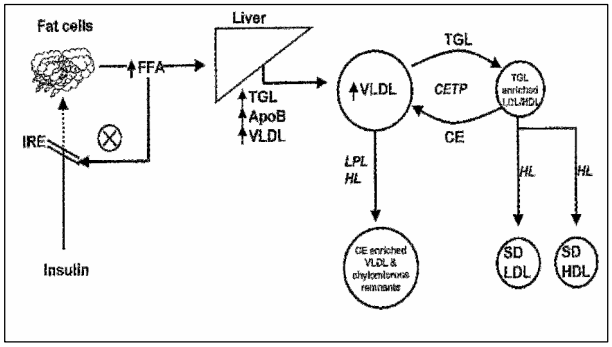

Figure 9. The mechanism relating insulin resistance with

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